PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 March 1999 (09.03.99)	
International application No. PCT/CA98/00648	**Applicant's or agent's file reference** DH/10648.31
International filing date (day/month/year) 02 July 1998 (02.07.98)	**Priority date** (day/month/year) 03 July 1997 (03.07.97)
Applicant ALLARD, France et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   02 February 1999 (02.02.99)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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PATENT COOPERATION TREATY

REC'D 26 OCT 1999
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
DH/10648.31		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA98/00648	02/07/1998	03/07/1997
International Patent Classification (IPC) or national classification and IPC A01N37/44		
Applicant UNIVERSITE DU QUEBEC A MONTREAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/02/1999	22.10.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Elliott, A Telephone No. +49 89 2399 8218

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA98/00648

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,3,4,7-12	as originally filed			
2,2a,5,6,6a,13	as received on	15/09/1999	with letter of	13/09/1999

Claims, No.:

1-37	as received on	15/09/1999	with letter of	13/09/1999

Drawings, sheets:

1/5-5/5	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18 as originally filed
	No:	Claims	19-24 as originally filed
Inventive step (IS)	Yes:	Claims	1-13 as originally filed
	No:	Claims	14-24 as originally filed
Industrial applicability (IA)	Yes:	Claims	1-24 as originally filed
	No:	Claims	-

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

In amended form, the application is directed to methods of:
(a) increasing or inducing the cold or freezing tolerance in a plant by acclimatising the plant to a temperature not lower than the coldest temperature that the plant can withstand for a time sufficient to induce cold or freezing tolerance in the plant and increasing the concentration of betaine or a derivative thereof in the plant to induce cold or freezing tolerance in the plant, the combined steps increasing or inducing the cold or freezing tolerance of the plant over and above the cold or freezing tolerance induced by each step alone (amended claims **1-25**);
(b) reducing the growth rate of a plant by treating the plant with a composition comprising betaine or derivative thereof at a dosage not lethal to the plant (amended claims **26-31**);
(c) stimulating and improving the germination rate of plant seeds at a temperature not lower than the coldest temperature that the plant seeds can withstand comprising administering to the seeds a betaine or betaine derivative composition and allowing the seeds to germinate at the said temperature (amended claim **32**);
(d) inducing or increasing cold tolerance in a plant sensitive to a temperature of about 0°C or higher by administering a composition comprising betain or derivative thereof to the plant prior to a decrease in temperature (amended claim **33**); and
(e) inducing or increasing cold or freezing tolerance in a plant by increasing the accumulation of a protein having the biological characteristics of WCOR410 in the plant (amended claim **34**).

The following documents are referred to in this report:

D1 : KISHITANI, S. ET AL, "Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants", PLANT, CELL AND ENVIRONMENT (1994), 17(1), 89-95
D2 : WO-A-96 41530 (CULTOR OY)
D3 : PERRAS MICHEL ET AL, "Synthesis of Freezing Tolerance Proteins in Leaves, Crown, and Roots during acclimation of Wheat", PLANT PHYSIOLOGY, vol. 89, 1989, pages 577-85
D4 : CA-A-2 104 142 A (SARHAN ET AL)
D5 : KOSTER, KAREN L. ET AL, "Solute accumulation and compartmentation during the cold acclimation of Puma rye", PLANT PHYSIOLOGY, 1992, 98(1), 108-113
D6 : DATABASE WPI Week 7349 Derwent Publications Ltd., London, GB; AN 73-75195U
D7 : US-A-4 360 465 (BUSCHMANN ET AL)
D8 : US-A-4 032 325 (KIDA TAKAO ET AL)
D9 : WO-A-96 41532 (CULTOR OY)
D10: WO-A-97 08951 (CULTOR OY)
D11: WO-A-95 35022 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION)

I Basis of the report

This report has been established as if some of the amendments had not been made since they have been considered to go beyond the disclosure as filed (Article 34(2)(b) and Rule 70.2(c) PCT):

Claim **1** of the application has been amended in such a way that its scope has been enlarged to include other methods of increasing the betaine (derivative) content of plants. It may be the case that other methods of increasing the betaine levels in plants have the same effect as administering the betaine but there is no such teaching in the application which says so. The amendment is therefore considered inadmissible in the eyes of the Examining Authority.

A further modification to claim 1 has been the removal of the passage "higher than about 0°C but" in step (a). Again this amendment is considered to enlarge the scope of the claim in a way not supported in the application as originally filed as temperatures below 0°C are now included under the scope of the claim. The amendment is hence also inadmissible.

New claim **8** is stated to be based upon the passage appearing in the description on page 6, line 14. The passage of the description is dealing solely with the method of the invention for two varieties of wheat. It is therefore considered inadmissible to generalise this narrow teaching for all plants understood to be treatable by the method of the application.

New claim **13** defines which betaines are meant to be understood by the more general term betaine by means of a formula, whereby the formula and its definitions have been generalised from the table of betaine compounds on pages 5 and 6 of the application as originally filed. The compounds falling under the scope of the formula, although they include those listed in the aforesaid table, are considered to include many compounds which cannot be said to be logically derivable from the few betaines listed in the table. The amendment to include this formula into the application is therefore considered inadmissible. New page 5 of the description is therefore also considered inadmissible on the same grounds.

With respect to new claim **17**, it is pointed out that golf turf is not a plant.

New claim **22** states that the freezing tolerance is increased by at least about 5°C whereas the claim upon which it is supposed to have been based (original claim 11) gives 6°C.

New claim **23** would appear to contain subject-matter not contained in the application as originally filed. Although the gene WCOR410 is mentioned in the application as originally filed, there is, however, no mention of the WCOR410 protein.

With the omission of the passage "by at least 30%" in new claim **26**, new claim **26**'s scope is considerably broader than that of original claim 14's, the claim upon which new claim **26** is based.

New claim **32** can be seen to be practically the same as original claim 22 but with the important difference that the passage "which is higher than about 0°C but" has been omitted in the new claim. This omission expands the scope of protection of the claim without any support for the amendment being present in the application as filed.

Finally and similarly to the point raised above with respect to new claim 23, claims **34**, **35** and **37** make reference to the protein WCOR410 which again is nowhere mentioned in the application as filed.

V Reasoned statement under Art 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

D1 discloses the correlation between the level of betaine accumulated during cold acclimatisation and the degree of freezing tolerance in leaves. The conclusion of D1 is that cold acclimatisation causes a build-up of betaine, which build-up being then responsible for an improved degree of freezing tolerance of leaves compared with leaves on plants which have not previously been subjected to a period of cold acclimatisation. The effect on barley plants is specifically exemplified.

D2 discloses the use of betaine to increase the yield of plants under both normal and

stress conditions. The betaine is applied exogenously with an adjuvant. Reference is made in particular to pages 1-10 and the claims of D2. In its discussion of the related art, D2 cites an article by Zhao et al (page 4, line 34-page 5, line 11) wherein it had been shown that glycinebetaine improved the freezing tolerance of alfafa seedlings.

D3 discusses the protein synthesis in leaves, crown and roots during the cold hardening of freezing tolerant winter wheat and freezing sensitive spring wheat.

D4 relates to three genes which were isolated from cold-tolerant wheat species and which are induced by cold temperatures.

D5 discusses the cold acclimatisation of puma rye whereby the accumulation of glycinebetaine and proline (amongst others) was monitored. The amount of glycinebetaine increases fourfold over a 4-week acclimatisation period. The amount of proline increased only in the 4th-6th weeks of acclimatisation. Under results, the notion that the accumulation of solutes during cold acclimatisation is involved in the increasing of the freezing tolerance is mentioned.

D6 discloses compounds for suppressing vertical plant growth. Betaines fall within the scope of the generic formula given in D6.

D7 discloses salts of α-aminoacetanilides for use as plant growth regulators. Certain of the compounds generically-disclosed are considered to fall under the scope of betaines.

D8 discloses a herbicidal composition comprising as the active compound amino acid higher alkyl esters (cf. column 2). Betaines fall under the scope of the generically-described compounds of D8.

D9 relates to the exogenous use of betaines to improve the yield of C-4 cereals, particularly under stress conditions.

D10 relates to the exogenous use of betaines to improve the yield of grain legumes particularly under stress conditions.

D11 discloses a method for treating seeds with betaines by either soaking the seeds in a betaine solution or coating the seeds with betaine prior to planting. The treatment enhances the growth of seedlings and provides the germinating seeds with protection against adverse conditions (cold, heat, drought and salinity).

i. **Novelty (Article 33(2) PCT)**

i.a. With its disclosure of the use of betaines to increase the yield of plants under stress conditions, D2 was to be seen as novelty-destroying to original claims **23** and **24**, increasing the yield under stress conditions being seen as synonymous to improving the tolerance under abiotic stress - if the yield is increased under stress, the tolerance of the plant under stress has obviously also been increased. The amended claims, however, do not include the subject-matter of original claims **23** and **24**, and so the new set of claims, although considered unallowable under Article 34(2)(b) PCT, does overcome this novelty objection.

i.b. The same comments as under point V.i.a are valid for documents D9-D11 in that they also disclose methods of treatment with betaines whereby the plant being treated is given in increased yield under stress conditions.

i.c. Document D6 was originally considered novelty-destroying to original claims **14** and **15** in that it specifically discloses a betaine derivative as a plant growth regulator. The applicant counter argued this objection by pointing out that D6 was concerned with the hydrochloride salts of betaines and not betaines themselves. The applicant puts the growth suppressing property of these compounds down to their acidity; he continues by stating that the concentration of the betaines used according to D6 is not sufficient to produce the desired growth suppressing effect of the present application. The fact that the compounds of D6 are used in the form of their hydrochloride salts is sufficient for D6 to be not considered prejudicial to the novelty of original claims **14** and **15** and subsequently new claim **26**.

i.d. Document D8 was originally seen to be novelty-destroying to original claims **19-21** in that it specifically discloses compounds falling under the scope of the general term betaine and having herbicidal activity. However, with the omission of the subject-matter of original claims **19-21** from the amended claimed subject-matter, document

D8 is not considered to affect the novelty of the newly-claimed subject-matter.

i.e. Document D9 was seen to be novelty-destroying to original claim **22** in that it discloses the use of betaine for increasing the germination rate of maize seeds. Similarly, example 4 of document D10 was also seen to be novelty-destroying to original claim **22** in that it shows the increase in germination rate of green bean seeds when betaine is applied to them. Document D11 was also clearly seen to be novelty-destroying to original claim **22** in that it is directed to a method of treating seeds with betaine to increase the germination rate of the seeds. The wording of new claim **32** is practically unchanged from that of original claim **22** and therefore new claim **32** remains lacking in novelty with respect to these 3 documents. The applicant's point about a combination of cold stress and betaine treatment being claimed in new claim **32** cannot be followed - new claim **32** does not stipulate a cold temperature but merely a temperature *not lower than the coldest temperature that the plant seeds can withstand.*

i.f With regard to new claim **33**, the wording of the claim appears to imply that the method merely involves the administering of the betaine (derivative) composition to increase/induce the plant cold or freezing tolerance. Such a method is already known from D2, D9-D11 where low temperatures are given as examples of stress conditions. New claim **33** therefore would appear to lack novelty with respect to the prior art.

i.g With respect to new claim **34**, its subject-matter would appear to be merely worded as the result to be achieved, especially considering the disclosure of document D4 which discloses genes found in cold-tolerant wheat, the genes being induced by low temperature and one of which being the WCOR410 gene. Going on to new claim **35**, this claim can be considered novel in that no single document links betaine concentration to the production of the gene WCOR410.

ii. **Inventive Step (Article 33(3) PCT)**

ii.a. The present application was seen to have 5 different objects:
(i) to provide a method of improving the cold or freezing tolerance of plants (original and amended claims);

(ii) to provide a method of reducing the growth rate of plants (original and amended claims);
(iii) to provide a method of killing a plant (original claims);
(iv) to provide a method of stimulating and improving the germination rate of plant seeds (original and amended claims); and
(v) to provide a method of improving the tolerance of a plant to abiotic stress (original claims).

ii.b. With respect to object (i), the closest prior art can be seen to be either a document disclosing the cold acclimatisation of plants causing a build-up of betaine in the plant whereby original claim **1** would be different from this in that it additionally includes the step of treating the plant with betaine or document disclosing the use of betaine to increase the cold or freezing tolerance of plants whereby present claim **1** would be different from this in that it additionally includes the step of cold acclimatisation. Both options are known in the art.

The fact that an optimal cold or freezing tolerance even higher than that achieved by each separate method can be achieved by combination of the methods could in no way have been predicted and thus the subject-matter of original claims **1-13** could be said to be the result of an inventive step.

If new claims **1-25** had been considered allowable under Article 34(2)(b) PCT, their subject-matter could also have been said to have been the result of an inventive step.

From their wording, new claims **33** and **34** have the same object (i):

New claim **33**, as indicated above under Novelty, would still appear to lack novelty with respect to the prior art, namely documents D2 and D9-D11.

As new claim **34** was considered to be a claim formulated along the lines of the result to be achieved as a result of D4's disclosure, inventive step examination is therefore based on new claim **35** which states the way in which protein having the biological characteristics of WCOR410, namely by increasing the concentration of betaine (derivative) in the plant. There can be seen to be nothing in the prior art which links betaine (derivative) concentration in a plant and increased WCOR410 genes in a

plant, and therefore the subject-matter of new claim **35** is seen to be the result of an inventive step (even though the claim's subject-matter was considered unallowable under Article 34(2)(b) PCT, cf. under point I above).

ii.c With respect to object (ii), document D6 was considered to provide a method for solving this object. The applicant's appraisal of D6 shows that it is most probably the acidic nature of the compounds of D6 which accounts for their growth regulating properties. The fact that, as the applicant states, the concentrations used in D6 are not sufficient for the betaine (derivatives) of the present application to act as plant growth regulators, seems to support this. The subject-matter of original claims **14-18** and hence amended claims **26-31** is therefore to be considered inventive with respect to D6 as D6's disclosure does not teach the subject-matter of the respective claims in the present application (but see point ii.d below).

ii.d. However, further with respect to object (ii), D7 generically discloses compounds falling under the scope of the term betaine which are used as plant growth regulators. Therefore the subject-matter of original claims **14-18** and hence amended claims **26-31** directed to those compounds generically disclosed in D7 is not considered inventive with respect to D7.

ii.e. With respect to object (iii), document D8 not only includes material which was seen as novelty-destroying to original claims **19-21** but also a more generic disclosure of compounds suitable as herbicides and including a number of betaines. Therefore the subject-matter of original claims **19-21** was not considered inventive as it was seen to be a selection from the teachings of D8. The subject-matter of original claims **19-21** is not included in the amended set of claims.

ii.f. With respect to object (iv) of the present application, original claim **22** was objected to as not being novel with respect to documents D9-D11. Original claim **22** was correspondingly not seen as inventive with respect to these documents. As new claim **32** was not considered to have overcome the novelty objection raised with respect to original claim **22**, the subject-matter of new claim **32** is also considered to lack as inventive step with respect to the prior art documents D9-D11.

ii.g. With respect to object (v) above, the subject-matter of claims **23** and **24** would

appear to have already been covered in prior art documents D2, D9-D11 (low temperature) and therefore nothing inventive could be seen in these claims. The subject-matter of original claims **19-21** is not included in the amended set of claims.

iii. Due to the inadmissibility of certain of the amended claims (cf. point I above), the presence or lack of novelty, inventive step and industrial applicability indicated in the report to which this separate is appended are meant to **refer to the claims as originally filed**.

VII Certain defects in the international application

On page 5, line 15 of the description as originally filed, the applicant gives a general formula for betaines. What is not clear from this formula and the definitions following the formula is what compounds are meant to be included by the definitions. The problem would appear to lie in the definitions given for x:
- firstly, when x were to equal 1, the N atom would not have a positive charge.
- secondly, when x were to equal 2, the N atom would have no charge and how the betaine could be a cyclic betaine is not at all clear.
- thirdly, when x were to equal 3.

Furthermore, the betaines given in Table 1 on the same page cannot all be seen to fall under the generic formula given for betaines.

As the generic formula supplied with the amendments during the international proceedings was not considered allowable, this objection is maintained.

In WO 96/41530, different compositions of betaine are disclosed for use in protecting wheat, potato and grapevines against adverse conditions including temperatures between 3°C and 30°C.

These references are silent with regards to freezing temperatures and cold acclimation.

In France Allard's thesis, it is taught that in the wheat cultivar Fredrick, cold acclimation for three days 6 °C/2 °C (day/night) combined with the addition of 1000 mM betaine resulted in the improvement in the freezing tolerance of the plants as tested by measuring the survival rate at -10 °C. The survival rate was shown to be 83%, whereas, when treated with only 1000mM betaine, the survival rate was 51%, when compared to controls. This reference does not teach on the optimal conditions for increasing freezing tolerance. The high amount of betaine used (1000mM) was shown to have a toxic effect to the plant since it was found that it produces chlorosis of the leaves. In addition the cultivar Fredrick having a LT_{50} (lethal temperature where 50% of the plants die) at -17 °C, testing the plants at -10 °C does not teach how more freezing tolerant is the plant when treated with the combined treatment. It further does not teach the effect on cultivars that genotypically exhibit less freezing tolerance. The decrease in plant growth rate is also described and it is shown to be directly proportional to the amount of betaine administered. When the maximal amount of 1000mM was applied to the cultivar Fredrick, a 29% decreased growth rate occurred when compared to control plants. Finally, in this document there were also teachings relating to the protein WCOR 410. It was taught that the protein WCOR 410 accumulates when plants are subjected to cold-acclimation or by increasing amounts of exogenous betaine. This reference however does not appear to teach a period of cold-acclimation which is sufficient for optimally inducing the expression of the *Wcor 410* gene and improving freezing tolerance.

Replaced by AR#34

In the patent publication CA 2,104,142, the present inventors disclose the isolation and sequence of three genes responsive to cold temperature. One of these genes is *Wcor410*. However what is not taught is that the protein WCOR410 is induced by betaine in a manner proportional to the amount of betaine applied and that this protein is involved in promoting freezing tolerance in some plants. It does not teach the benefits of combining cold acclimation and betaine administration.

of regrowth and greening in the betaine-treated area. This result reflects a greater and healthier winter survival of betaine-treated turf.

Figure 4b). Close up caption showing the betaine-treated area (upper view) and the non-treated control (bottom).

Figures 5a) and b). Another example of the effect of betaine on winter survival and regrowth in early spring. The betaine treatment was the same as in Figure 4 except that the application was started earlier in previous fall. Both areas were aerated at the same time. Notice the higher turf density of the betaine-treated area.

Figure 5a). Betaine-treated.

Figure 5b). Control sprayed with water.

Betaine refers to amino acids where the nitrogen is fully or partly methylated. Betaines are natural products present in plants and animals with a probable function as an osmolyte regulator that protect the cell from osmotic stress. Betaine have the general formula:

$$(CH_3)_x - N^+ - (CH_2)_y - COO^-$$

where x may be 1 and preferably 2 for cyclic betaine or 3 for straight chain betaines, and y is at least 1. The most common betaine is a glycine derivative where the three methyl groups are attached to the nitrogen of a glycine molecule.

Other betaines that are known of which some that are available commercially are presented in Table 1.

TABLE I

NAMES	OTHER NAMES
Glycinebetaine	Oxyneurin, betaine
β-alaninebetaine	Homobetaine
2-trimethylamino-6-ketoheptanoate	
Prolinebetaine	Stachydrine
Proline	
N-methyl-L-proline	
Trans-4-hydroxy-N-methyl-L-proline	
Cis-3-hydroxy-N-methyl-L-proline	
(-)4-hydroxyproline betaine	Betonicine
(+)4-hydroxyprolinebetaine	Turicine
3-hydroxyprolinebetaine	3-oxystachydrine
Histidinebetaine	Herzynine, Ercinine
Tryptophanbetaine	Hypaphorine

2-mercaptohistidine-betaine	Ergothioneine
Pipecolabetaine	Homostachydrine
Nicotinic acid betaine	Trigonelline

Using two wheat cultivars that differ in their levels of freezing tolerance (FT), the role of endogenous betaine was investigated during cold acclimation. In addition, studies on the effect of an exogenous application of betaine on FT alone and in combination with cold acclimation, on the expression of low temperature-responsive genes and on photosynthetic activity have been conducted.

To determine if betaine accumulation is associated with increased FT, the betaine contents were determined in two wheat varieties differing in their FT (cv Glenlea, LT_{50} (lethal temperature for 50% of the plants) of -8°C and cv Fredrick, LT_{50} of -17°C). In both cultivars, betaine content decreases during growth at the non-acclimated temperature of 24/20°C while it increases during growth at the cold-acclimating conditions of 6/2°C. The basal betaine level is 30% higher in the more tolerant cultivar Fredrick before cold acclimation (8.5 µmol/g FW in Fredrick compared to 6.5 µmol/g FW in Glenlea). At the end of the acclimation period (where maximal LT_{50} has been reached) cv Fredrick has accumulated 21.3 µmol/g FW of betaine compared to 15.3 µmol/g FW for cv Glenlea. On a dry weight basis, cv Fredrick has accumulated 106.5 µmol/g DW compared to Glenlea which has accumulated 82.7 µmol/g DW. This result suggests that the increase in betaine content is associated with the development of FT of the two cultivars. A similar increase in betaine was correlated with the FT of different barley cultivars (11). If we calculate the contribution of betaine to the total osmolality of the cell, we find that betaine accounts for only 3.6% and 4.5% of the osmolality after 30 days of cold acclimation for Glenlea and Fredrick respectively. This result demonstrates that betaine contribution to the total osmolality is very low. However, as suggested by Wyn Jones *et al* (12), such a low concentration would require compartmentation in order to play a significant role as osmoprotectant. Studies performed by Matho *et al* (13) have shown that betaine is excluded from vacuoles of spinach leaf cells and is mostly found in the cytoplasm and chloroplasts. It was estimated that betaine concentration can reach 300 mM in spinach (14) and *Sueda* (8) chloroplasts when plants are submitted to salt stress. This concentration is approximately 20 fold greater than the average betaine leaf concentration. Betaine compartmentation was not determined in wheat but if we consider a similar concentration factor in the chloroplasts during cold acclimation, the actual concentration of betaine could be very significant. Since we have estimated that betaine accounts for 4.5% of the osmolality in cold-acclimated Fredrick, a twenty fold higher concentration of betaine in the chloroplast would mean that betaine contributes for approximately 90% of the chloroplasts' osmolality (or 612 mOsm). Such a

24. A. G. Ivanov, M. Krol, D. Maxwell, N. P. A. Huner. *FEBS Lett.* **371**: 61-64 (1995).
25. M. Perras, F. Sarhan. *Plant Physiol.* **89**: 577-585 (1989).

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of increasing cold or freezing tolerance in a plant, which comprises the steps of:

- acclimating said plant to a temperature higher than about 0°C but not lower than the coldest temperature that said plant is capable to withstand, for a time sufficient to induce an optimal cold or freezing tolerance, in said plant, and

- administering a composition comprising betaine or a derivative thereof to said plant, in a dosage regimen sufficient to induce the same or different optimal cold or freezing tolerance in said plant;

whereby combined steps of cold-acclimating and administering betaine or derivative thereof increase cold or freezing tolerance of said plant over and above the optimal cold or freezing tolerance induced by each step alone.

2. A method as set forth in claim 1, wherein said dosage regimen is non-toxic to said plant.

3. A method as set forth in claim 1 or 2, wherein said plant is selected from gramineae and grasses.

4. A method as set forth in claim 3, wherein said gramineae is barley or wheat.

5. A method as set forth in claim 4, wherein said time for cold-acclimating is about four weeks.

6. A method as set forth in any one of claims 1 to 4, wherein betaine derivative is glycine betaine.

7. A method as set forth in claim 5, wherein betaine derivative is glycine betaine.

8. A method as set forth in claim 7, wherein said dosage regimen is growing said plant in the presence of a solution of glycine betaine having a concentration lower than about 500 mM.

9. A method as set forth in claim 8, wherein said concentration is about 250 mM.

10. A method as set forth in claim 9, wherein said plant is the spring wheat variety Glenlea, in which the optimal freezing tolerance, expressed as the temperature where fifty percent of a plant population die (LT_{50}) is about -8 °C for each step alone.

11. A method as set forth in claim 10, wherein the increase of freezing tolerance is by about 6°C to reach a LT_{50} of about -14°C.

12. A method as set forth in claims 10 or 11, wherein the optimal freezing tolerance induced by said each step alone or in combination is due at least in part to an increased expression of the gene *Wcor410*.

13. A method as set forth in claim 9, which further results in improving photosynthetic capacity and overall physiology of said plant at cold temperature.

14. A method of reducing the growth rate of a plant by at least 30%, thus having a growth-retarding effect which comprises the step of treating said plant with an effective dosage regimen of a composition comprising betaine or derivative thereof which is not lethal to said plant.

15. A method as set forth in claim 14, wherein said dosage regimen of betaine or derivative thereof is not toxic to said plant.

16. A method according to claim 15, wherein said plant is golf course grass.

17. A method as set forth in claim 14, wherein said plant is spring wheat variety Glenlea.

18. A method as set forth in claim 17 wherein said dosage regimen is growing said plant in the presence of 500 mM of glycine betaine for four days which results in a growth rate reduction by about 75%.

19. A method of killing a plant, which comprises the step of treating said plant with a composition comprising betaine or derivative thereof, thus having a herbicidal effect thereon at a lethal dosage regimen.

20. A method according to claim 19 wherein said plant is a dicotyledon.

21. A method according to claim 20, wherein said plant is selected from the group consisting of dandelion, canola, alfalfa, strawberry and tobacco.

22. A method of stimulating and improving the germination rate of plant seeds at a temperature which is higher than about 0°C but not lower than the coldest temperature that said plant seeds can withstand, which comprises the steps of administering to said seeds a composition comprising betaine or derivative thereof at an effective dosage regimen, and allowing said seeds to germinate at said temperature.

23. A method of improving tolerance of a plant to an abiotic stress, which comprises the step of administering a composition comprising an effective amount of betaine or derivative thereof.

24. A method according to claim 23, wherein said stress is selected from the group consisting of drought, salinity, cold and freezing temperatures.